



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/10, 15/67, 15/83, C12Q 1/68,</b> <b>C12N 15/82 // A01H 3/00</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 99/36516</b> <b>(43) International Publication Date:</b> 22 July 1999 (22.07.99)
<b>(21) International Application Number:</b> PCT/US99/01164 <b>(22) International Filing Date:</b> 15 January 1999 (15.01.99)  <b>(30) Priority Data:</b> 09/008,186 16 January 1998 (16.01.98) US  <b>(71) Applicant (for all designated States except US):</b> BIOSOURCE TECHNOLOGIES, INC. [US/US]; 3333 Vaca Valley Parkway, Vacaville, CA 95688 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> DELLA-CIOPPA, Guy [US/US]; 814 Derry Circle, Vacaville, CA 95688 (US). ERWIN, Robert, L. [US/US]; 336 Summerfield Drive, Vacaville, CA 95687 (US). FITZMAURICE, Wayne, P. [US/US]; 1218 Las Encinas Court, Vacaville, CA 95687 (US). HANLEY, Kathleen, M. [US/US]; 1230 Red Oak Court, Vacaville 95687 (US). KUMAGAI, Monto, H. [US/US]; 809 Plum Lane, Davis, CA 95616 (US). LINDBO, John, A. [US/US]; 143 Sundance Drive, Vacaville, CA 95688 (US). McGEE, David, R. [US/US]; 1990 Marshall Road, Vacaville, CA 95687 (US). PADGETT, Hal, S. [US/US]; 638 Blossom Oak Court, Vacaville, CA 95687 (US). POGUE, Gregory, P. [US/US]; 419 Trillick Court, Vacaville, CA 95688 (US).	<b>(74) Agent:</b> HALLUIN, Albert, P.; Howrey & Simon, 1299 Pennsylvania Avenue, N.W., Box 34, Washington, DC 20004 (US).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 16 March 2000 (16.03.00)	

**(54) Title:** METHOD OF DETERMINING THE FUNCTION OF NUCLEOTIDE SEQUENCES AND THE PROTEINS THEY ENCODE BY TRANSFECTING THE SAME INTO A HOST

**(57) Abstract**

The present invention provides methods for rapidly determining the function of nucleic acid sequences by transfecting the same into a host organism to effect expression. Phenotypic and biochemical changes produced thereby are then analyzed to ascertain the function of the nucleic acids which have been transfected into the host organism. The invention also provides methods for silencing endogenous genes by transfecting hosts with nucleic acid sequences to effect expression of the same. The present invention also provides methods for selecting desired functions of RNAs and proteins by the use of virus vectors to express libraries of nucleic acid sequence variants. Moreover, the present invention provides methods for inhibiting an endogenous protease of a plant host.

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# INTERNATIONAL SEARCH REPORT

Int. .onal Application No

PCT/US 99/01164

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/10 C12N15/67 C12N15/83 C12Q1/68 C12N15/82  
//A01H3/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SABLOWSKI, R.W.M., ET AL.: "expression of a flower-specific Myb protein in leaf cells using a viral vector causes ectopic activation of a target promoter" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 92, July 1995, pages 6901-6905, XP002118431 see the whole document ---	1-18, 23-28, 56-58
X	WO 95 34668 A (BIOSOURCE TECH INC) 21 December 1995  see the whole document ---	1-18, 21-27, 29-33, 35-37, 56-58
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

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- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- \*&\* document member of the same patent family

Date of the actual completion of the international search

15 October 1999

Date of mailing of the international search report

27. 01. 2000

Name and mailing address of the ISA

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# INTERNATIONAL SEARCH REPORT

Intel onal Application No

PCT/US 99/01164

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 01375 A (ICI PLC) 7 February 1991 see page 3, line 8 - line 14 ---	51-53, 56-58
P,X	WO 98 36083 A (ANGELL SUSAN MARY ;BAULCOMBE DAVID CHARLES (GB); PLANT BIOSCIENCE) 20 August 1998  see page 3, line 8 - line 14; example 8 ---	1-18, 22-27, 29-33, 35,36, 51-53, 56-58
T	BAULCOMBE , D.C.: "fast foward genetics based on virus-induced gene silencing" CURRENT OPINION IN PLANT BIOLOGY, vol. 2, 1999, pages 109-113, XP002118432 see the whole document line 14; example 8 -----	1-18, 21-37

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 99/01164

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1, 51-53, 56-58 (partially); 2-50, 117 (completely)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

**1. Claims: 1,51-53, 56-58 partially, 2-50,117 completely**

A method to determine the function of a nucleic acid sequence or members of a cDNA- or EST-library in an organism by utilizing a viral expression vector and observing changes which result from the expression of the nucleic acid; a method of silencing endogenous genes using such viral vector; a method for the construction of a cDNA library.

**2. Claims: 51-53, 56-58 partially, 54,55,59-74 completely**

A method to determine the function of a nucleic acid sequence by altering the genome of a host organism by transposon mutagenesis and observing changes which result from the subsequent expression of the nucleic acid of interest.

**3. Claims: 75-77 completely**

A method to identify a gene product target of an inhibitor by identifying the gene product that corresponds to a conditional lethal mutation.

**4. Claims: 78-92 completely**

A method for constructing an infectious viral vector and method for infecting a plant host with such a vector.

**5. Claims: 93-97 completely**

A method for inhibiting an endogenous protease of a plant by applying a compound that induces the production of an inhibitor of said protease.

**6. Claim : 98 completely**

A method for improving the expression of an exogenous nucleic acid sequence in a plant comprising interspecific hybridisation.

**7. Claims: 99-114 completely**

A method for optimizing the function of a nucleic acid sequence in a plant by expressing variant forms of the nucleic acid sequence utilizing a viral expression vector.

**8. Claim : 115 completely**

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

A method of increasing the representation of a nucleic acid sequence in a viral expression library by propagating said library in the absence of *E. coli*.

**9. Claim : 1 partially; 116 completely**

A method to determine the function of a gene wherein one or more reporter genes are fused to one or more promoters in a viral expression vector.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/01164

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